Figure 1

Lane 1: RNA ladder
2: intact rRNA
3: intact rRNA +
   SB328636
4: TS43 + TS44 nts 207-504
5: TS44 + TS45 nts 509-801
6: TS45 + TS46 nts 806-1020
7: TS46 + TS47 nts 1025-1282
8: TS47 + TS48 nts 1287-1465
9: TS48 + TS49 nts 1470-1671
10: TS49 + TS50 nts 1676-1966
11: TS50 + TS36 nts 1971-2306
12: TS36 + TS40 nts 2313-2607
13: TS40 + TS42 nts 2614-2820

Figure 2

Figure 3

Radiolabel in RNaseH 23S rRNA Fragments

SB325636
SB352408

% of total dpm

nucleotides

509- / 801, 806- / 1020, 1025- / 1282, 1287- / 1465, 1470- / 1671, 1676- / 1966, 1971- / 2306, 2313- / 2607, 2614- / 2820

Figure 4

Chiral

SB-352408

Chiral
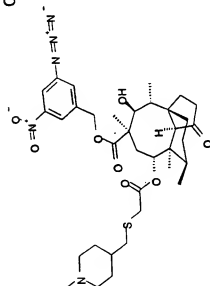
SB-328636

Figure 5

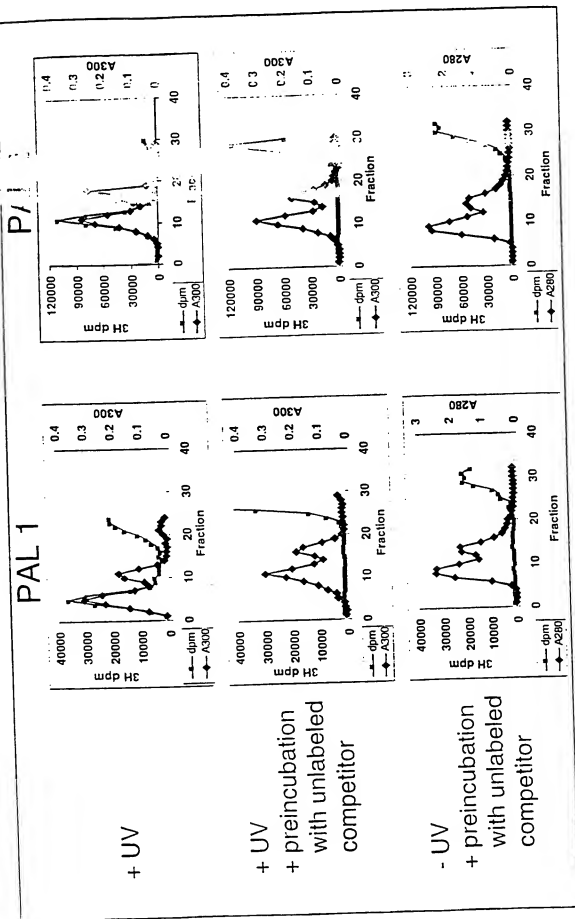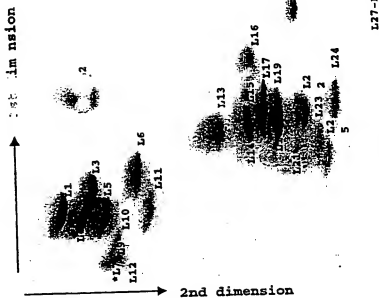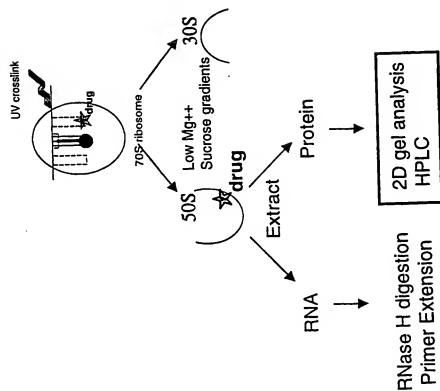|	PAL-1	PAL-2
RLB IC50 (uM)	TBD	0.54
S30 fmet IC50 (uM)		
Organism	MICs (ug/mL)	
E. coli DC0	>64	>64
E. coli DC2	64	8
H. influenzae Q1	>64	0.5
M. catarrhalis 1502	0.25	≤ 0.06
E. faecalis I	>64	>64
S. aureus Oxford	2	0.25
S. pneumoniae R6	16	≤ 0.06
S. pyogenes CN 10	2	<l 0.06

Figure 6.

Figure 7.

Figure 8.

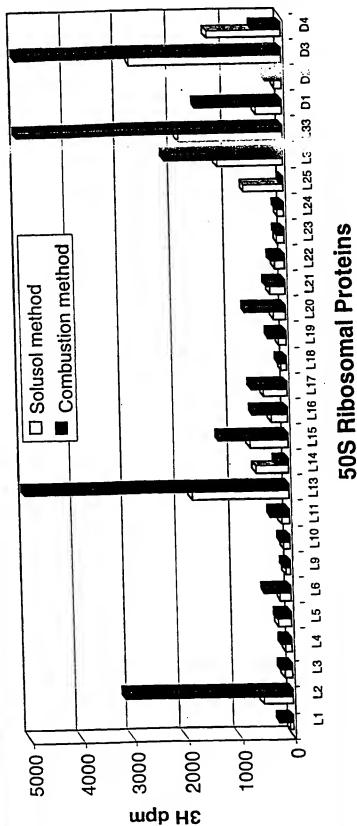* L7, L12, L27, and L28 are seen with larger protein loads.

Figure 9.

PAL-1
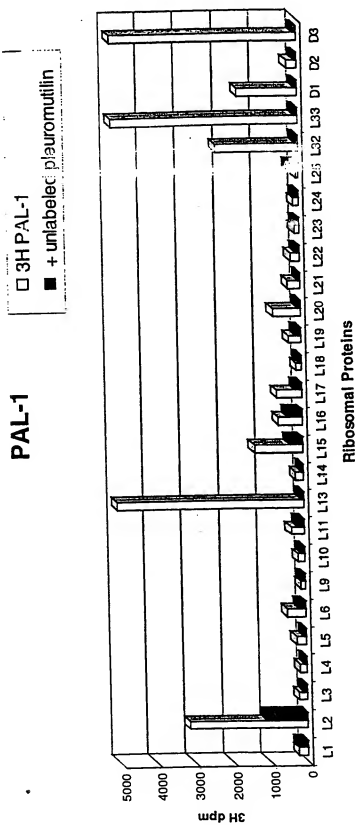
Legend:
☐ 3H PAL-1
■ + unlabeled pleuromutilin

y-axis: 3H dpm (0, 1000, 2000, 3000, 4000, 5000)

x-axis: Ribosomal Proteins (L1 L2 L3 L4 L5 L6 L9 L10 L11 L13 L14 L15 L16 L17 L18 L19 L20 L21 L22 L23 L24 L25 L32 L33 D1 D2 D3)
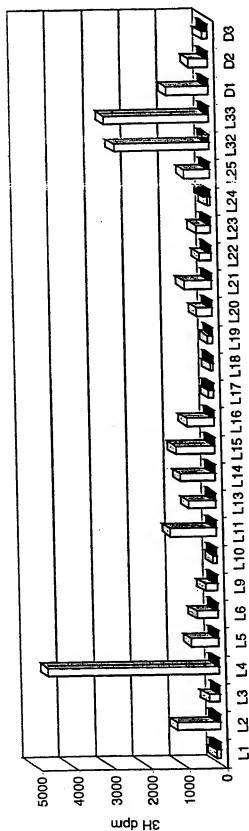
Figure 10.

Figure 11.

Figure 12.

Figure 13.

FIGURE 14

Fig 15.

³H Radioligand

Unbound ³H Radioligand

Count ³H Radioligand retained on filter

Figure 16..

S30 lysate+ Buffers, Energy Source

[³H]fmet-tRNAmet

+/- Pleuromutilin

Incubate

TCA precipitate polypeptide
Filter

Count ³H f-met labeled
peptides retained on filter
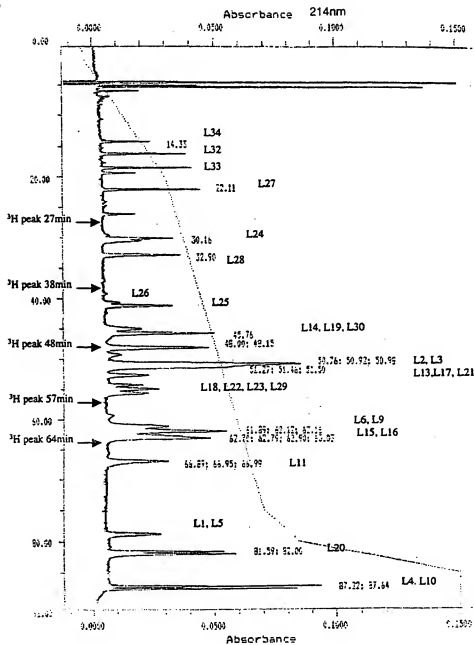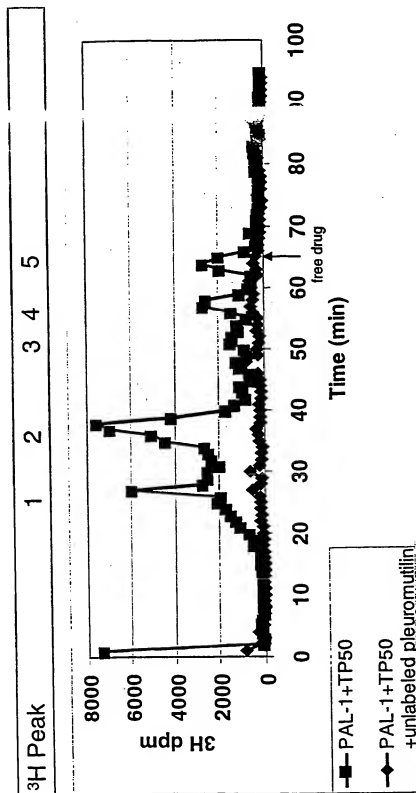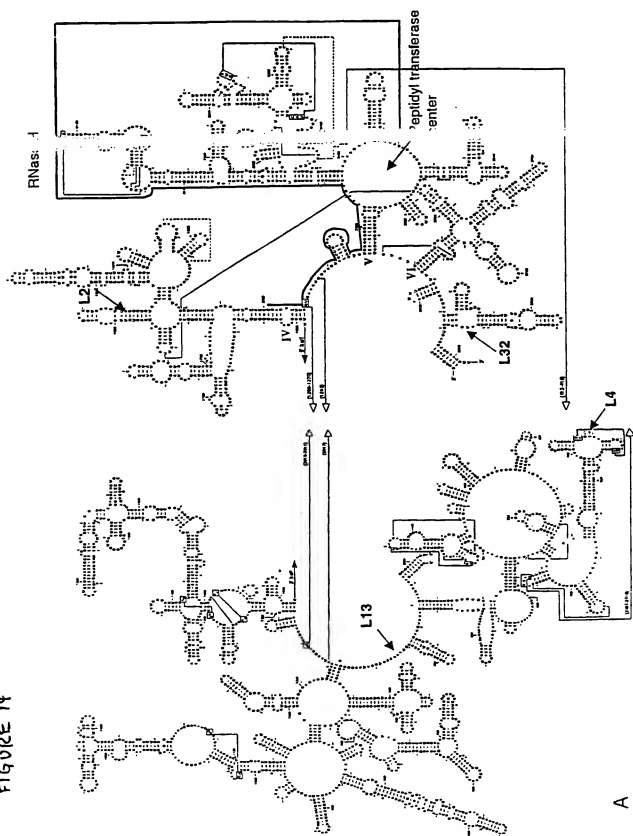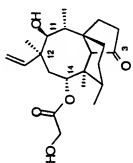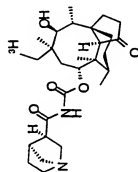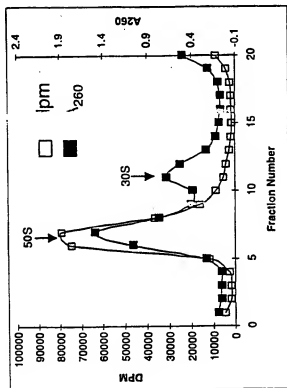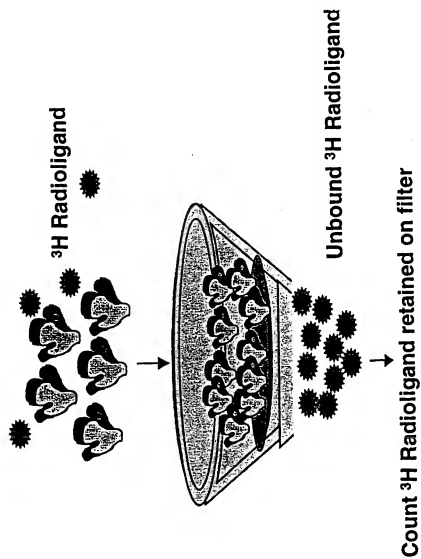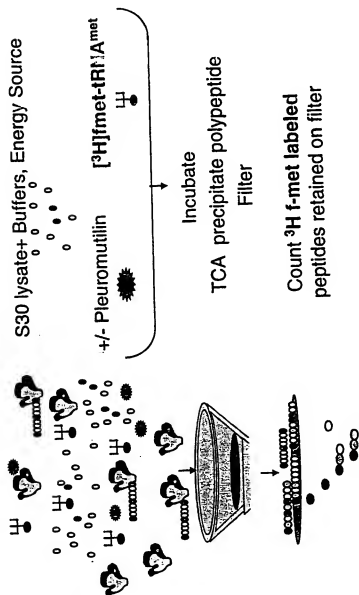
Figure 17.

Figure 18.

Figure 19.

- ● A, <0.06 ug/mL
- ■ B, <0.25 ug/mL
- ▲ C, <0.25 ug/mL
- ▼ D, <0.25 ug/mL
- ● E, 2 ug/mL
- ■ F, 2 ug/mL
- ▲ G, 2 ug/mL
- ● H, >64 ug/mL
- ■ I, 8 ug/mL
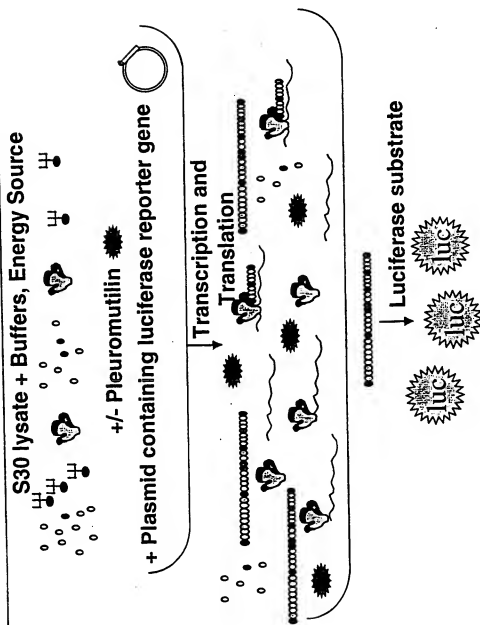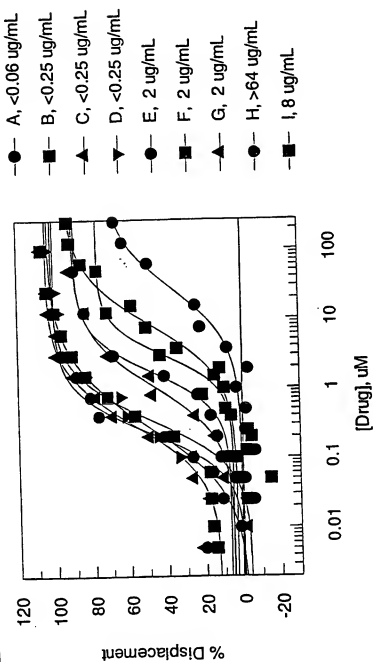
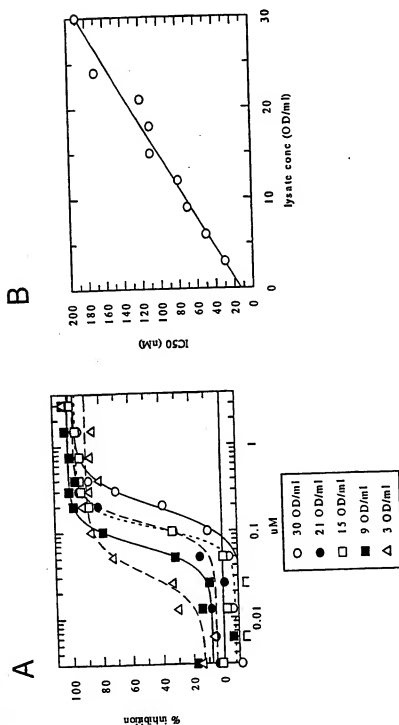| SB Compound	S. aureus Oxford MICs (ug/mL)	Radioligand Binding (RLB) IC50 (uM)	S30 [3H] fmet IC50 (uM)	S30 Coupled Transcription/Translation IC50 (uM)
A	≤ 0.06	0.57 +/- 0.04	0.25±0.03	0.5 +/- 0.1
J	≤ 0.06	0.99 +/- 0.09	0.18±0.02	1.4 +/- 0.2
K	0.125	0.43 +/- 0.09	0.19±0.03	0.45 +/- 0.08
L	0.125	0.7 +/- 0.1	0.5±0.1	0.58 +/- 0.09
M	4	7.4 +/- 0.5	1.4±0.5	1.5 +/- 0.2
N	2	1.3 +/- 0.2	0.28±0.05	2.3 +/- 0.4
E	2	1.8 +/- 0.4	1.6±0.08	1.8 +/- 0.2
F	2	2.7 +/- 0.4	nd	0.4 +/- 0.1
G	2	1.1 +/- 0.5	0.27±0.07	0.7 +/- 0.08
O	4	8±1	1.4±0.7	1.8 +/- 0.3
P	4	0.8 +/- 0.3	0.47±.08	1.2 +/- 0.2
H	> 64	12±3	nd	49±7
I	8	18±4	50%at50 uM	1.1±0.2
Q	> 4	2.5 +/- 0.3	1.2±0.2	2.6 +/- 0.3
R	> 64	> 250	> 250	> 250
S	32	24±9	6±2	31 +/- 4
Correlation versus MICs (R value from linear regression	xx	0.69	0.99	0.79
Correlation versus RLB (R value from linear regression fit)	xx	xx	0.98	0.99

Fig 20.

Figure 21.

Figure 22.

A
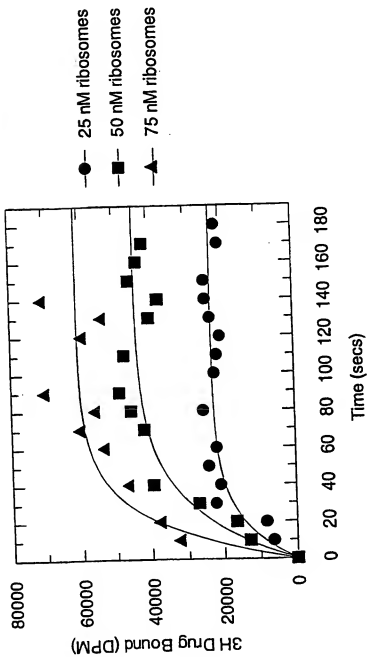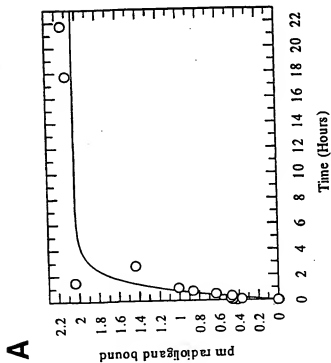
B

SB compound	S. aureus MIC (ug/mL)	$k_{dissociation}$	$t_{1/2}$ (min)
A	≤ 0.06	0.38	> 8 x 60
E	2	1.1	108
F	2	0.9	40
M	4	0.64	46
Q	>4	0.72	60
U	4		58

Figure 23.